



PATENT  
Docket No.: 19603/871 (CRF D-1792A)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Bomshiteyn et al.

Serial No. : 09/089,894

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Filed : June 3, 1998

For : BETULINOL DERIVATIVES

Examiner:  
D. Lukton

Art Unit:  
1653

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**DECLARATION OF BRIJ B. SAXENA UNDER 37 CFR § 1.132**

Assistant Commissioner for Patents  
Washington, D.C. 20231  
Box:

Dear Sir:

I, BRIJ B. SAXENA, declare:

1. I received a Bachelor of Science degree in Physics, Chemistry, and Biology from University of Lucknow in 1949, a Masters degree in Physiology from University of Lucknow in 1951, a Ph.D. in Biochemistry and Biology from University of Lucknow in 1954, a Doctor of Science in Biochemistry and Biology from University of Muenster in 1957, and a Ph.D. in Endocrinology and Biochemistry from University of Wisconsin in 1961.

2. I am currently Director of the Division of Reproductive Endocrinology in the Department of OB/GYN at Cornell University Medical College, Harold and Percy Uris Professor of Reproductive Biology at Cornell University Medical College, and Professor of Endocrinology at Cornell University Medical College.

3. I am currently a member of the editorial board for Proceedings of the Society for Experimental Biology and Medicine and Journal of Immunoassay.

4. I am a joint inventor for the above-identified application.

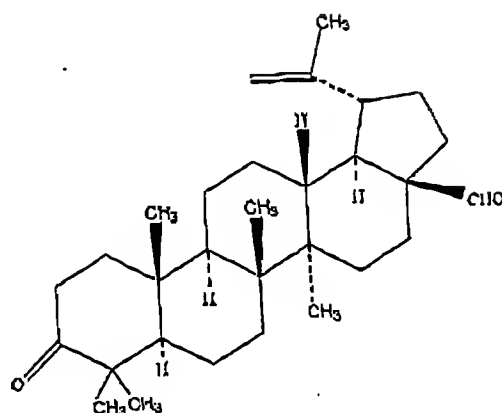
5. I am submitting this Declaration to describe experiments conducted on betulinaldehyde and a betulinal diether which show that betulinal diethers, as claimed in my above-identified application, can be used to treat patients suffering from cancer.

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## 6. Betulinol aldehyde

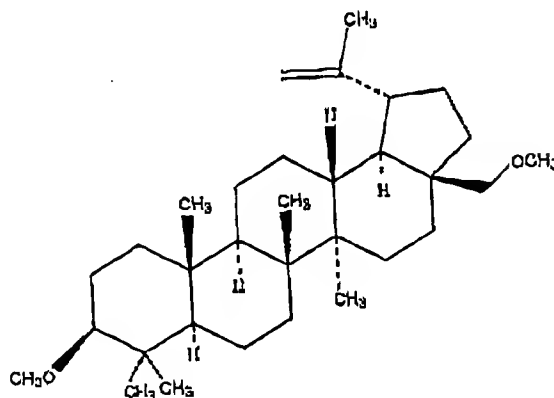


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and a betulinol diether



were submitted to the National Cancer Institute for evaluation for anti-cancer activity. The compounds were subjected to a 3-cell line panel consisting of NCI-H460 (lung), MCF7 (breast), and SF-268 (central nervous system). This 3-cell line, one-dose assay has been in use by the National Cancer Institute for several years for the evaluation of combinatorial libraries and has proven to be an effective pre-screen.

7. In the 3-cell line, one dose assay, each cell line is inoculated and pre-incubated on a microtiter plate. Test agents (e.g., betulinol aldehyde or betulinol diether) are then added at a single concentration and the culture incubated for 48 hours. End point determinations are made with sulforhodamine B, a protein-binding dye. Results for each test agent are reported as the percent of growth of the treated cells when compared to the

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untreated control cells. Compounds which reduce the growth of any one of the cell lines to 32% or less (negative numbers indicate cell kill) are active and are passed on for evaluation in the full panel of 60 cell lines over a 5-log dose range.

8. Both betulinol aldehyde and the betulinol diether were found to exhibit anti-cancer activity by the National Cancer Institute's 3-cell line, one dose primary anti-cancer assay, as shown in Table 1, below:

Table 1. Evaluation in the 3-cell line, one dose primary anti-cancer assay.

Sample	Concentration	NCI-H460 (lung) - growth %	MCF7 (breast) - growth %	SF-268 (CNS) - growth %	Activity
betulinol aldehyde	$1 \times 10^{-4}$ Molar	11	32	73	Active
betulinol dimethyl ether	$1 \times 10^{-4}$ Molar	20	3	-5	Active

In particular, betulinol aldehyde reduced the growth of the NCI-H460 (lung) and MCF7 (breast) cell lines to 32% or less. The betulinol diether reduced the growth of the NCI-H460 (lung) and MCF7 (breast) cell lines to 20% or less. Accordingly, betulinol diethers, as claimed in my above-identified application, can be used to treat patients suffering from cancer.

9. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

May 25, 2001  
Date: \_\_\_\_\_

Brij B. Saxena  
Brij B. Saxena

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